RESEARCH ARTICLE

Barrier-to-Autointegration Factor 1 (BAF/BANF1) Promotes Association of the SETD1A Histone Methyltransferase with Herpes Simplex Virus Immediate-Early Gene Promoters

Hyung Suk Oh,[a] Paula Traktman,[b] David M. Knipe[a]

Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA[a]; Department of Microbiology and Molecular Genetics, Wisconsin Medical College, Milwaukee, Wisconsin, USA[b]

ABSTRACT **We have shown previously that A-type lamins and intranuclear localization of the herpes simplex virus (HSV) genome are critical for the formation of the VP16 activator complex on HSV immediate-early (IE) gene promoters in murine cells, which implies a critical role for lamin A and its associated proteins in HSV gene expression. Because barrier-to-autointegration factor 1 (BAF/BANF1) has been thought to bridge chromosomes to the nuclear lamina, we hypothesized that BAF might mediate viral genome targeting to the nuclear lamina. We found that overexpression of BAF enhances HSV-1 replication and knockdown of BAF decreases HSV gene expression, delays the kinetics of viral early replication compartment formation, and reduces viral yield compared to those in control small interfering RNA-transfected cells. However, BAF depletion did not affect genome complex targeting to the nuclear periphery. Instead, we found that the levels of a histone-modifying enzyme, SETD1A methyltransferase, and histone H3 lysine 4 trimethylation were reduced on IE and early (E) gene promoters in BAF-depleted cells during HSV lytic infection. Our results demonstrate a novel function of BAF as an epigenetic regulator of HSV lytic infection. We hypothesize that BAF facilitates IE and E gene expression by recruiting the SETD1A methyltransferase to viral IE and E gene promoters.**

IMPORTANCE **The nuclear lamina is composed of lamin proteins and numerous lamina-associated proteins. Previously, the chromatin structure of DNA localized proximally to the lamina was thought to be characterized by heterochromatin marks associated with silenced genes. However, recent studies indicate that both heterochromatin- and euchromatin-rich areas coexist on the lamina. This paradigm suggests that lamins and lamina-associated proteins dynamically regulate epigenetic modifications of specific genes in different locations. Our goal is to understand how the lamina and its associated proteins regulate the epigenetics of genes through the study of HSV infection of human cells. We have shown previously that A-type lamins are critical for HSV genome targeting to the nuclear lamina and epigenetic regulation in viral replication. In this study, we found that another lamina-associated protein, BAF, regulates HSV gene expression through an epigenetic mechanism, which provides basic insights into the nuclear lamina and its associated proteins' roles in epigenetic regulation.**

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Address correspondence to David M. Knipe, david_knipe@hms.harvard.edu.

Herpes simplex virus (HSV) has a large (150-kbp) double-stranded DNA (dsDNA) genome that is transcribed and replicated in the host cell nucleus. Histones are not associated with viral DNA in the virion, but upon the entry of the viral genome into the nucleus, nucleosomes are rapidly associated and chromatin-modifying enzymes are recruited to viral promoters (1–3) to regulate viral gene expression. Herpesvirus immediate-early (IE) gene transcription depends on the HSV virion protein 16 (VP16)-induced transactivator complex (VP16/Oct-1/HCF-1), which recognizes an enhancer core element, ATGCTAATG ARAT (where R is a purine), in IE gene promoters (4–9). VP16 interacts with multiple general transcription factors (10–15) and subunits of Mediator in the RNA polymerase II holoenzyme to regulate IE gene transcription (16, 17). HCF-1 recruits multiple transcription factors, including Sp1 (18), GABP (19), and FHL2 (20), and epigenetic modifiers, including SETD1A (21), KDM1A (LSD1) (22), and KDM4s (JMJD2s) (23), to facilitate IE gene transcription (9, 24).

At early times postinfection, viral replication compartments (RCs) form at the nuclear periphery (25, 26), and we have shown that this phenotype depends on A-type lamins in murine cells (27). Interestingly, the VP16-induced transactivator complex also develops near the nuclear periphery (28). In lamin A/C knockout (*LMNA*$^{-/-}$) murine embryo fibroblast (MEF) cells, RCs are relocalized from the nuclear periphery to the nucleoplasm, heterochromatin marks are increased on IE gene promoters, and nuclear localization of the VP16/HCF-1 complex is lower in the nucleus than in normal MEF cells (27, 28). In addition, HSV-1 replication is lower in lamin B1 knockout (*LMNB1*$^{-/-}$) MEFs than in normal MEFs (29), implying a critical role for the lamina and its associ-

ated proteins in viral gene transcription. However, it is not clear which other host factors in the nuclear lamina contribute to HSV transcription. We therefore hypothesized that lamina-associated proteins might also be important for targeting of the HSV-1 genome to the nuclear periphery and its transcription. Barrier-to-autointegration factor 1 (BAF/BANF1) is thought to bridge chromosomes to the nuclear lamina and recruit epigenetic modifiers to the nuclear lamina (30, 31); therefore, BAF could mediate viral genome targeting to the nuclear lamina to facilitate viral replication.

The human *BANF1* gene encodes the 89-amino-acid BAF protein, which is highly conserved among metazoans (32–34). BAF binds to dsDNA and forms a homodimer, which has raised the idea of BAF bridging DNAs to form a higher order of chromatin structure (35–37). BAF also interacts with multiple cellular proteins, including LAP2-emerin-MAN1 (LEM) domain-containing proteins, lamins, histones, DNA damage response proteins, transcription factors, and epigenetic modifiers (38–42). Although the localization of BAF is cell type dependent, BAF localizes, in general, in both the cytoplasm and the nucleus with an enrichment near the interior of the nuclear envelope (43). BAF is a substrate of cellular vaccinia-related kinase 1 (VRK1) and VRK2 and protein phosphatase 2 (PP2) and PP4 (44–48). Modification of the phosphorylation status of BAF is critical for mitosis and nuclear reassembly (45, 49), which could explain the essential role of BAF during embryonic stages of development (34, 50, 51). Recently, mutant BAF (Ala12Thr) from hereditary Nestor-Guillermo progeria syndrome (52, 53) showed the phenotype of abnormal nuclear shape similar to A-type lamin mutant cells from progeria patients (54).

BAF was originally identified as a factor that can inhibit intramolecular integration of retroviruses (55, 56), and it was later shown to be a component of proviral preintegration complexes (57). BAF can inhibit vaccinia virus replication, and these activities depend mainly on the DNA-binding capability of BAF, which is related to the phosphorylation status of BAF (58). These events occur in the cytoplasm, where the viral genomes are released and replicate. However, BAF might affect viral replication differently in the nucleus, where gene expression is tightly regulated by the cellular environment. A recent study showed that a mutant BAF protein, but not WT BAF, can inhibit HSV replication in murine cells (59); however, the effect of wild-type (WT) BAF on HSV replication in human cells remains unclear.

Here we tested our hypothesis by evaluating the functional roles of BAF in HSV lytic infection. Our results show that BAF depletion reduced HSV gene expression, delayed the kinetics of viral early RC formation, and reduced the viral yield compared to those of control small interfering RNA (siRNA)-transfected cells. Interestingly, BAF depletion did not induce relocalization of RCs developing near the nuclear periphery but instead reduced the levels of a euchromatin mark on IE gene promoters. Therefore, these results support our hypothesis that, in addition to A- and B-type lamins, other lamina-associated proteins play roles in HSV replication but the mechanisms by which they promote HSV-1 replication might be different from the lamin A-dependent mechanism.

RESULTS

BAF is required for efficient HSV-1 replication. To investigate the effect of BAF on HSV-1 replication, we depleted BAF in HeLa cells by using siRNAs. We transfected HeLa cells twice with a pool of BAF-specific siRNAs or nontarget (NT) siRNAs as a control and evaluated the levels of BAF. BAF was reduced to the limit of detection from 4 to 6 days posttransfection (dpt) (Fig. 1A). Under these conditions, we observed a 20 to 30% reduction in the number of BAF-depleted HeLa cells compared with that of mock- or NT siRNA-transfected cells. To determine the effect of the reduced level of BAF on HSV-1 replication, we performed viral replication assays with BAF-depleted HeLa cells at a high (10 PFU/

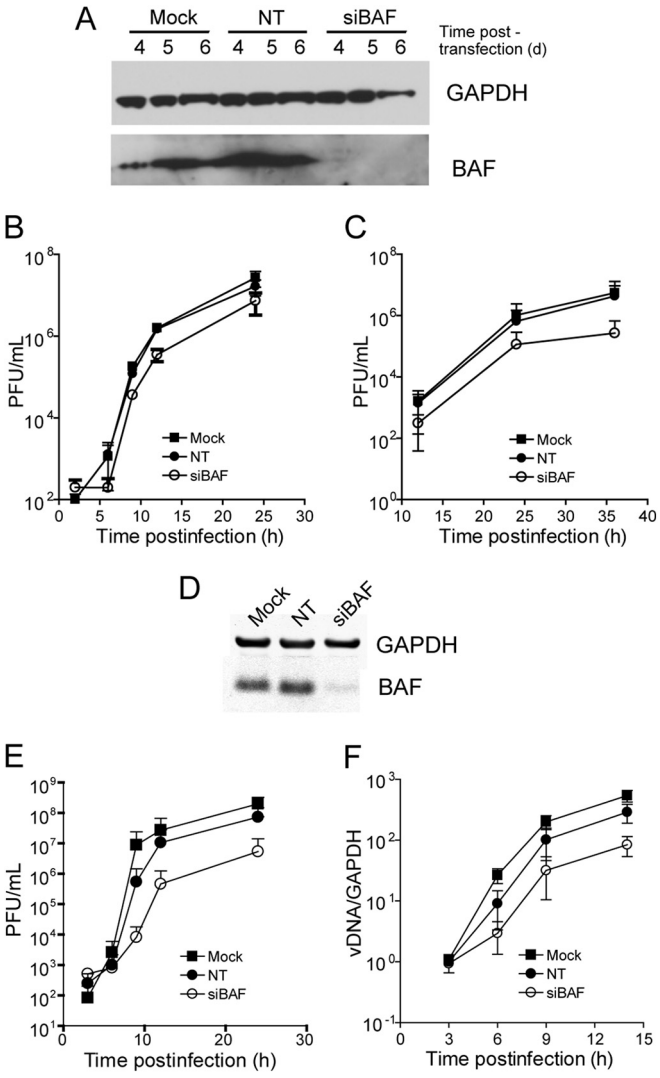

FIG 1 Effect of BAF on HSV replication. (A) Pools of NT control or BAF-specific siRNAs were transfected into HeLa cells, and BAF and GAPDH protein levels were analyzed by immunoblotting with the specific antibodies. Mock-treated samples were from cells with no siRNA transfection. GAPDH was detected as a loading control. d, days. (B and C) HSV-1 growth curves at high and low MOIs, respectively. HeLa cells were infected with HSV-1 at an MOI of 10 (B) or 0.05 (C) and harvested at the postinfection times indicated, and viral yield was determined by plaque assay. (D) NT control or BAF-specific siRNAs were transfected into HFF cells, and protein levels were analyzed as described above. Mock-treated samples were from cells with no siRNA transfection. (E and F) HFF cells were infected with HSV-1 at an MOI of 10 and harvested at the postinfection times indicated, and viral yields (E) and vDNA levels (F) were determined by plaque assay and quantitative PCR, respectively. Results shown are means and standard deviations from three independent experiments.

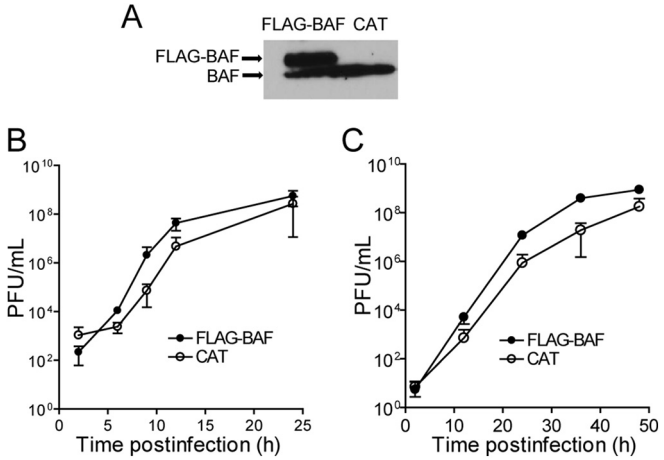

FIG 2 Effect of BAF overexpression on HSV-1. (A) FLAG-BAF was detected in FLAG-BAF-expressing CV-1 cells by immunoblotting with anti-BAF antibody. CAT-expressing CV-1 cells were used as a control. (B and C) CV-1 cells stably expressing FLAG-BAF were infected with HSV-1 at an MOI of 10 (B) or 0.05 (C) and harvested at the postinfection times indicated, and viral yields were determined by plaque assays. Results shown are means and standard deviations from three or more independent experiments.

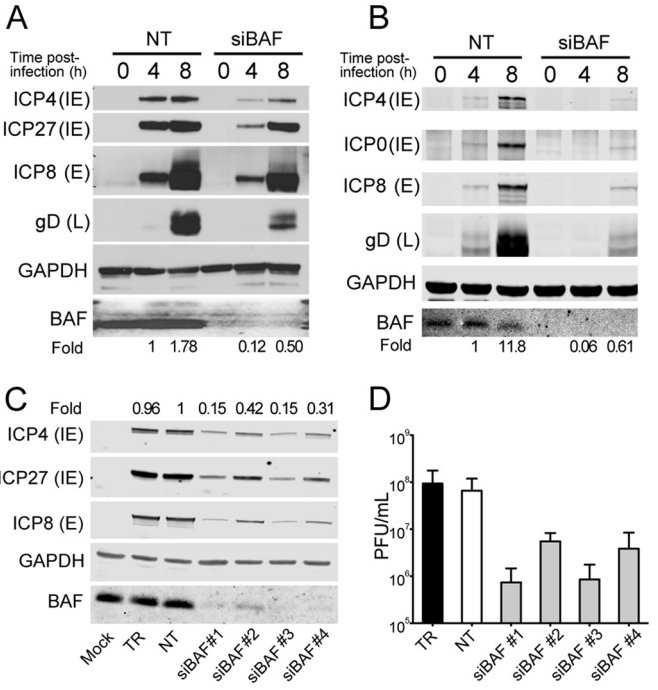

FIG 3 Effect of BAF on viral protein expression. HeLa (A) or HFF (B) cells were transfected with NT control or BAF-specific siRNA and infected with HSV-1 at an MOI of 10. Cells were harvested at the postinfection times indicated, and IE, E, and L gene proteins were detected by immunoblotting with the specific antibodies. GAPDH was detected as a loading control, and BAF depletion was confirmed with a BAF-specific antibody. Relative (fold) differences in ICP4 levels are shown below the immunoblots. (C and D) HFF cells were transfected with NT control or individual BAF-specific siRNAs (siBAFs 1 to 4) and infected with HSV-1 at an MOI of 10. Cells were harvested at 6 (C) and 12 (D) hpi. (C) Proteins were detected by immunoblotting, and relative (fold) differences in ICP4 levels are shown above the immunoblot. (D) Viral yields were determined by plaque assays. Mock-treated samples are from cells without virus infection, and TR is a sample from transfection reagent-treated cells infected with HSV-1. Results shown are means and standard deviations from three independent experiments.

cell) or low (0.05 PFU/cell) multiplicity of infection (MOI). At the high MOI, we observed a 2- to 5-fold lower viral yield at various times postinfection in BAF-specific siRNA-transfected HeLa cells than in controls, mock-transfected (Mock) or NT siRNA-transfected HeLa cells (Fig. 1B). When we infected HeLa cells at the low MOI, the reduction of the HSV-1 yield was more significant, approximately 10- to 20-fold, at various times postinfection (Fig. 1C). These results suggested that BAF contributes to efficient HSV-1 replication in HeLa cells. The nearly normal yields at the high MOI suggested that the low-MOI effect was not largely due to toxic effects of the BAF-specific siRNAs on the cells. To confirm the effect of BAF, we tested the role of BAF in human foreskin fibroblast (HFF) cells. As described above, we depleted BAF by using BAF-specific siRNAs, confirmed the reduction of BAF (Fig. 1D), infected HFF cells at an MOI of 10, and determined the viral yield (Fig. 1E) and viral genome (viral DNA [vDNA]) accumulation (Fig. 1F). We observed 10- to 20-fold lower viral yield (Fig. 1E) and 3- to 6-fold less vDNA accumulation (Fig. 1F) at various times postinfection in the BAF-depleted cells than in control mock-transfected (Mock) or NT siRNA-transfected HFF cells. These results indicated that the contribution of BAF to efficient HSV-1 replication is a general phenotype in different human cell lines rather than a cell line-specific phenotype.

To further test the role of BAF in HSV-1 replication, we overexpressed FLAG-BAF in a stably expressing CV-1 cell line (FLAG-BAF). In FLAG-BAF CV-1 cells, the level of FLAG-BAF was approximately 3-fold higher than that of endogenous BAF, and the endogenous BAF levels were similar in FLAG-BAF and control chloramphenicol acetyltransferase (CAT)-expressing CV-1 cells (Fig. 2A). We infected the FLAG-BAF or CAT CV-1 cells with HSV-1 at a high (10 PFU/ml) or low (0.05 PFU/ml) MOI and determined the viral yields at various times postinfection. At the high MOI, we observed 28-fold higher HSV-1 yields in FLAG-BAF CV-1 cells at 9 h postinfection (hpi) than in CAT CV-1 cells, but by 24 hpi, there was only a 2-fold difference (Fig. 2B). At the low MOI, we observed a 20-fold greater viral yield in FLAG-BAF cells than in CAT CV-1 cells at 36 hpi and a 5-fold difference at 48 hpi (Fig. 2C). Therefore, ectopic expression of BAF accelerated HSV-1 replication and increased the final viral yield by 2- to 5-fold, consistent with BAF being a positive factor in HSV-1 replication.

BAF is required for optimal IE gene expression. To investigate the stage of infection that was affected by BAF, we examined the accumulation of viral proteins. We transfected HeLa (Fig. 3A) or HFF (Fig. 3B) cells with either NT control or BAF-specific siRNA twice, and at 3 dpt, we infected the cells at an MOI of 10. We harvested the cells at various times postinfection and measured the levels of IE infected-cell protein 4 (ICP4) and ICP27, the early (E) ICP8 protein, and the late (L) gD protein by immunoblotting. The levels of both IE proteins were lower at 4 and 8 hpi in BAF siRNA-transfected cells than in NT control siRNA-transfected cells (Fig. 3A and B). The accumulation of the E (ICP8) and L (gD) proteins also decreased. In BAF siRNA-transfected cells, the level of BAF was below the limit of detection and the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) remained constant to 8 hpi. These results supported the idea that BAF promotes the expression of IE proteins.

To ensure that the pool was specific for BAF, we next examined the levels of viral proteins and viral yields following the depletion

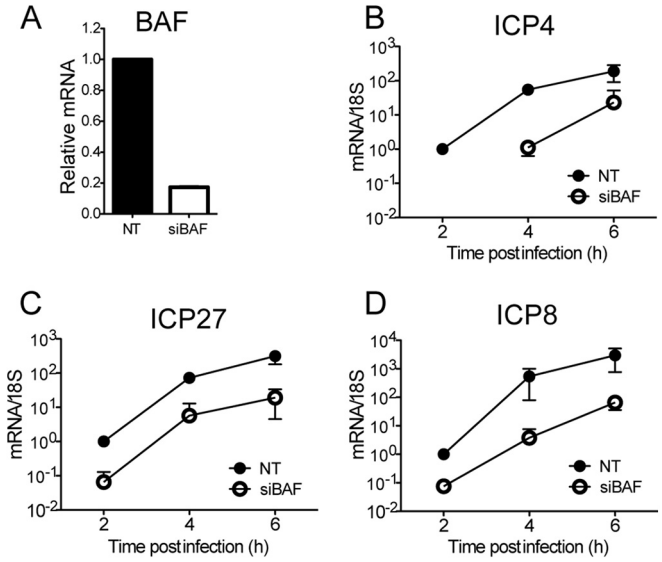

FIG 4 Effect of BAF on viral transcript levels. HFF cells were transfected with NT control or BAF-specific siRNA and infected with HSV-1 at an MOI of 10. (A) Knockdown efficiency was confirmed by quantitative RT-PCR. (B to D) Cells were harvested at the postinfection times indicated, total RNAs were prepared, and IE (*ICP4* [B] and *ICP27* [C]) and E (*ICP8* [D]) gene mRNA levels were measured by quantitative RT-PCR. The mRNA levels were normalized to 18S rRNA. Results shown are means and standard deviations from three independent experiments.

of BAF with individual BAF-specific siRNAs from the pool. We transfected HFF cells with each individual BAF-specific siRNA, infected the cells, and harvested them at 6 hpi (Fig. 3C) and 12 hpi (Fig. 3D) as described above. Depletion of BAF by each individual BAF-specific siRNA reduced the levels of viral proteins (Fig. 3C) and viral yields (Fig. 3D). The level of BAF protein was reduced by all four individual BAF-specific siRNAs, and the depletion of BAF with siBAF 2 appeared less efficient than that with the other siRNAs (Fig. 3C), which correlated with the reduced viral protein levels (Fig. 3C) and viral yields (Fig. 3D). These results further supported our conclusion that the reduction of HSV-1 gene expression and viral production resulted from the depletion of BAF rather than from off-target effects of the siRNAs.

To determine the effect of BAF on viral transcription, we measured viral RNA levels in HFF cells by reverse transcription (RT)-PCR. When we depleted BAF with siRNAs, we observed >5-fold less BAF RNA in the cells transfected with the BAF-specific siRNAs than in NT control siRNA-transfected cells (Fig. 4A). BAF depletion caused a 10-fold reduction in *ICP4* gene transcripts (Fig. 4B), *ICP27* gene transcripts (Fig. 4C), and *ICP8* gene transcripts (Fig. 4D). These results indicated that BAF promotes viral lytic gene transcription.

BAF does not affect nuclear entry or association of VP16 or viral DNA. Previous studies showed that lamin A promoted the nuclear accumulation of VP16 and its association with IE gene promoters in murine cells (28). Therefore, it was possible that other lamina-associated proteins, including BAF, could affect VP16 accumulation in the nucleus. We first evaluated the entry of input virion VP16 into the nucleus. We infected HFF cells with VP16-green fluorescent protein (GFP) HSV-1 at a high MOI used previously to detect input VP16 (28) in the presence of cycloheximide and separated the cells into nuclear and cytoplasmic fractions. Immunoblotting of the subcellular fractions for VP16-GFP showed that the localization of VP16 to the infected cell nucleus was equivalent in the presence or absence of BAF (Fig. 5A). We also looked at the accumulation of viral DNA genomes in the nuclei of HFF cells, and knockdown of BAF did not affect the nuclear accumulation of viral DNA genomes (Fig. 5B). Therefore, it appeared that BAF did not affect the nuclear localization of VP16 or the viral genome.

We then looked at the effect of BAF on VP16 association with IE gene promoters. HFF cells were transfected with NT control- or BAF-specific siRNA and then infected with HSV-1 DG1, which expresses VP16-GFP. The cells were harvested at 2 hpi, and chromatin immunoprecipitation (ChIP) was performed with an anti-GFP antibody. Similar amounts of viral IE (*ICP4* and *ICP27*) gene promoter sequences were immunoprecipitated with VP16-GFP from BAF-depleted and control cells (Fig. 5C).

We also tested whether BAF plays a role in VP16 transactivator complex formation and viral genome targeting to the lamina. Viral genome complexes (ICP4 foci) develop asymmetrically in the nuclei of cells at the edge of a developing plaque (26), but defective VP16 transactivator complex formation or depletion of lamin A led to reduced ICP4 focus development at the periphery of the nucleus (27, 28). BAF is thought to bridge cellular chromosomes to the nuclear lamina (30, 31). Therefore, we hypothesized that BAF might recruit and/or bridge viral genomes to the nuclear lamina. If BAF affected viral genome targeting and/or VP16 transactivator complex formation to the nuclear lamina, then depletion of BAF should reduce asymmetric genome complex development. To test this hypothesis, we examined the localization of ICP4 in cells at the edge of a developing plaque. We infected BAF-depleted HFF cells with HSV-1 at an MOI of 0.05 to 0.1, incubated the cells in medium to allow plaque formation, fixed the cells at 40 hpi, and performed an indirect immunofluorescence assay with an antibody specific to ICP4 to visualize genome complexes. BAF depletion led to a reduced number of plaques (results not shown), but the numbers and sizes of the genome complexes revealed by ICP4 immunofluorescence were similar (Fig. 5D). Furthermore, there were no significant changes in the location of ICP4 in BAF-depleted cells compared to that in control cells (Fig. 5D), indicating that BAF played no apparent role in targeting of the viral genome to the nuclear lamina. These results indicated that BAF likely plays a role in the steps after viral genome positioning to the nuclear lamina.

BAF is not critical for heterochromatin exclusion from RCs. As described above, we had found that BAF promotes the expression of viral IE proteins. Consistent with this, the formation of viral RCs was delayed and reduced in BAF siRNA-transfected HFF cells compared to that in NT siRNA-transfected cells (Fig. 6). Interestingly, the H3K9me3 heterochromatin mark association was excluded from ICP8-labeled RCs in cells transfected with NT or BAF-specific siRNAs. Therefore, in contrast to previous results with lamin A/C (28), we found that BAF did not noticeably affect the exclusion of heterochromatin from the RCs. It is noteworthy that depletion of BAF in HFF cells altered the shape of their nuclei (Fig. 6), as observed for HeLa cells (43), but the alteration was less noticeable in HFF cells.

BAF promotes accumulation of a euchromatin mark on IE genes. Overexpression or depletion of BAF can alter the profiles of global histone modifications (60), and BAF is reported to interact with various histone-modifying factors (41); thus, BAF may be

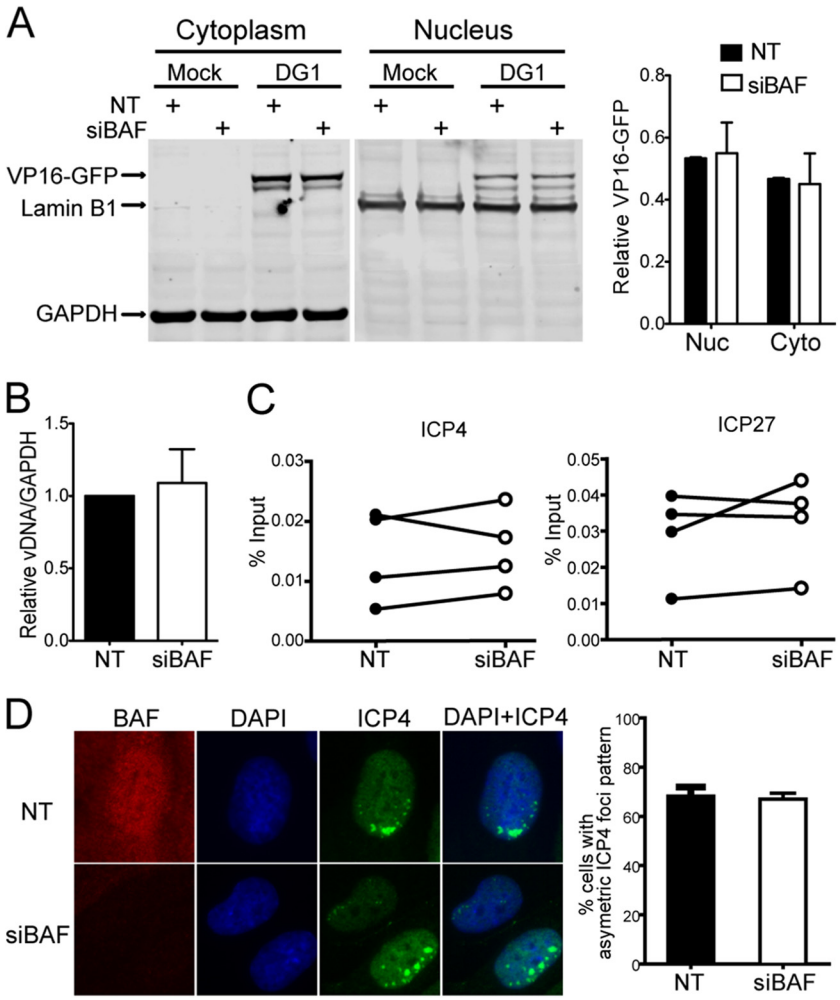

FIG 5 Effect of BAF on nuclear entry of VP16, viral DNA, and localization to RCs. HFF cells were transfected with NT control or BAF-specific siRNA and infected with HSV-1 DG1 at an MOI of 100. (A) The cells were harvested at 3 hpi and separated into cytoplasmic (Cyto) and nuclear (Nuc) fractions. VP16-GFP was detected with anti-GFP antibody (left). GAPDH and lamin B1 were used as loading controls. The relative levels of VP16-GFP in the cytoplasmic and nuclear fractions were quantified with Image Studio Lite (right). The histogram shows the mean values and standard deviations from two independent experiments. (B) HFF cells were transfected with NT control or BAF-specific siRNA and infected with HSV-1 at an MOI of 10. The cells were harvested at 2 hpi, nuclei were isolated, and vDNA levels were measured by quantitative PCR. The histogram shows the mean values and standard deviations from three independent experiments. (C) ChIP assays were performed to determine the levels of VP16-GFP on IE gene promoters. HFF cells transfected with NT control or BAF-specific siRNA were infected with HSV-1 DG1 at an MOI of 5 and fixed at 3 hpi. ChIP was performed with an anti-GFP antibody, and the amounts of the *ICP4* and *ICP27* gene promoters immunoprecipitated were determined by quantitative PCR. Lines connect values of individual experiments. (D) HFF cells were transfected with NT control or BAF-specific siRNA, infected with HSV-1 at an MOI of 0.1, and fixed at 40 hpi, and an indirect immunofluorescence assay was performed with antibodies specific for BAF and ICP4. The nuclei were stained with DAPI. The histogram represents the mean values and standard deviations from three independent experiments ($n = >50$ per individual experiment).

involved in epigenetic modification of the HSV-1 genome during productive infection. To investigate the potential role of BAF as an epigenetic regulator of HSV-1 replication, we performed ChIP to determine whether histone loading and/or modifications were affected by the reduction of BAF. We transfected HeLa cells with NT control or BAF-specific siRNA, infected them with the WT virus, harvested them at various times postinfection, and performed ChIP with antibodies specific for histone H3, H3K9me3, or H3K4me3. As reported previously (3, 61), the levels of H3 and its H3K9me3 and H3K4me3 modifications on the *ICP27* gene were high at 2 to 4 hpi and decreased at later times postinfection. We did not observe any significant changes in the levels H3 or H3K9me3 on the *ICP27* gene from cells transfected with BAF-specific siRNA compared to those from cells transfected with NT control siRNA from 2 to 8 hpi (Fig. 7A and B, top). However, we observed a 2- to 8-fold reduction of the H3K4me3 modification level in the *ICP27* gene promoter at 2 to 4 hpi in cells transfected with BAF-specific siRNA compared to that in NT control siRNA-transfected cells (Fig. 7C, top). To confirm the reduced H3K4me3 modification in the *ICP27* promoter region at an early time postinfection, we repeated the ChIP assays at 2 hpi as described above. The levels of H3K4me3 were 2-fold lower at 2 hpi in the cells transfected with BAF-specific siRNA than those in NT control siRNA-transfected cells (Fig. 7C, bottom), but the levels of H3 and H3K9me3 were not significantly different at the same time postinfection (Fig. 7A and B, bottom). These results were consis-

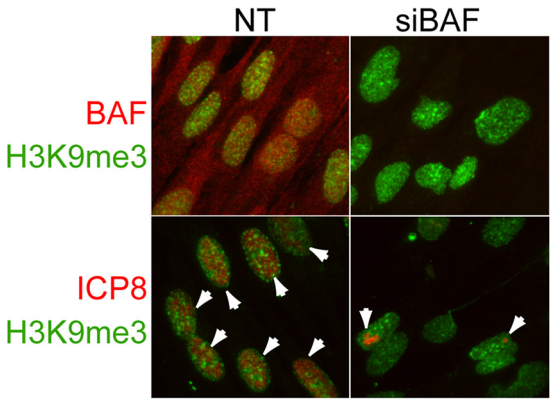

FIG 6 Knockdown of BAF reduces the size and numbers of viral RCs, but heterochromatin is excluded from the RCs. HFF cells were transfected with NT control or BAF-specific siRNA and infected with HSV-1 at an MOI of 10. At 8 hpi, the cells were fixed and processed for indirect immunofluorescence assays with antibodies specific for BAF (red in top row), ICP8 (red in bottom row), and H3K9me3 (green). Arrows indicate ICP8-stained RCs.

tent with our observation of H3K9me3 exclusion from HSV-1 RCs (Fig. 6) and suggested that BAF increases the H3K4me3 euchromatin mark on HSV-1 IE gene promoters.

To investigate the functional specificity of BAF for HSV, we first evaluated the association of BAF with viral and cellular gene promoters. We transfected HeLa cells with a hemagglutinin (HA)-BAF or control plasmid, infected the cells with HSV-1, harvested them at 2 hpi, and performed ChIP assays with an HA-specific antibody. We observed that BAF associated with the IE (I*CP0*, *ICP4*, and *ICP27*), E (*ICP8*), L (*gC*), and *GAPDH* gene promoters (Fig. 7D). We then looked at the association of SETD1A with IE gene promoters in BAF-depleted cells because the SETD1A methyltransferase is known to be responsible for trimethylation of H3K4 for HSV replication (62). We observed a 2- to 4-fold reduction in the level of SETD1A associated with IE and E gene promoters but a lesser change on the L (*gC*) gene promoter and no change on *GAPDH* from the cells transfected with BAF-specific siRNA compared to the NT control siRNA-transfected cells (Fig. 7E). BAF depletion did not affect the levels of SETD1A protein expression (results not shown). In total, these results argued for a functional role for BAF in the regulation of the H3K4me3 level on viral IE and E gene promoters by recruitment of SETD1A.

BAF colocalizes with RCs. BAF binds to dsDNA nonspecifically; thus, BAF might associate with the HSV-1 genome during viral gene transcription and replication to regulate gene expression. To investigate the association of BAF with the HSV-1 genome during replication, we examined the localization of BAF during times of viral DNA synthesis. We infected HFF cells with HSV-1 and detected BAF and RCs with antibodies specific for BAF and ICP8, respectively. In mock-infected HFF cells, we found BAF in both the cytoplasm and the nucleus (Fig. 8A). From 4 hpi (results not shown) onward, we observed the accumulation of BAF in HSV-1 RCs, increasing until at least 8 hpi (Fig. 8A). This result suggested that BAF could have a direct functional role in viral protein expression and replication. Because BAF colocalized with ICP8, we attempted to identify any viral proteins associated with BAF. We infected HeLa cells transiently expressing FLAG-BAF with DG1 VP16-GFP recombinant HSV-1, lysed them, and immunoprecipitated FLAG-BAF with an anti-FLAG antibody. We observed that ICP8 coimmunoprecipitated with FLAG-BAF, but neither ICP4 nor VP16-GFP was detected (Fig. 8B). Reverse coimmunoprecipitation also showed an association between FLAG-BAF and ICP8 (Fig. 8B). These results showed that BAF localizes to RCs and associates with ICP8, which implies a potential second stage at which BAF might function in the viral replication cycle.

DISCUSSION

Epigenetic mechanisms play important roles in the regulation of gene expression, and viruses have evolved to hijack this system to transcribe their own genes (24). HSV genome complexes and RCs form near the nuclear lamina inside the nucleus of an infected cell (25–27), and this is linked to epigenetic regulation of the viral genome. We have shown that A-type lamin knockout cells showed reduced viral gene transcription and replication because of altered epigenetic regulation (27). A VP16 mutant HSV-1 strain showed an epigenetic phenotype similar to that found in lamin A/C knockout (*LMNA*$^{-/-}$) MEF cells (28), suggesting that the nuclear lamina plays a role in targeting of the genome in the nucleus, assembly of the VP16 transactivator complex, and promotion of euchromatic modifications on viral chromatin. Here we show that the BAF dsDNA-binding protein, which is thought to bridge DNA to nuclear lamins, promotes HSV-1 transcription. Overexpression of BAF increases viral yields, and depletion of BAF reduces viral transcription. Depletion of BAF does not affect the level of VP16 in the nuclei of infected human cells or association of VP16 with IE gene promoters, which is different from the phenotypes of *LMNA*$^{-/-}$ cells. Interestingly, depletion of BAF reduces the level of the H3K4me3 euchromatin mark histone and its corresponding histone methyltransferase SETD1A (KMT2F) on viral IE gene promoters, arguing that BAF regulates HSV gene expression epigenetically but that its mechanism is distinct from the VP16-lamin A/C-dependent mechanism.

Role of BAF in viral gene expression. We showed that depletion of BAF reduced viral yields in HeLa and HFF cells, arguing that BAF has a positive role in HSV replication. When we depleted BAF in HeLa and HFF cells, both cell types grew slower than NT control siRNA-transfected cells but continued to grow nonetheless. Although endogenous BAF was difficult to detect by immunoblotting, the levels of BAF transcripts were still present at 10% of the control siRNA levels, indicating that the residual BAF expression may have allowed the cells to survive during the knockdown period. This was observed previously with lentivirus-mediated knockdown of BAF in CV-1 cells (63). To exclude any potential side effect from siRNA-mediated knockdown of BAF and confirm the positive functional role of BAF in HSV replication, we also tested viral gene expression in CV-1 cells (CV-1–FLAG-BAF) that overexpressed BAF. We found that the HSV yield was higher in CV-1–FLAG-BAF cells than in control CV-1-CAT cells, supporting a positive role for BAF in HSV replication.

BAF is thought to have positive or negative effects on gene expression in different systems. Homodimerized BAF molecules are thought to bridge DNAs and cause chromatin compaction and accumulation of heterochromatin, thereby silencing certain genes (34, 36, 37). On the other hand, overexpression of BAF has been reported to induce euchromatin marks (60), and BAF binds transcription factors (41). Depletion of BAF in mouse embryonic stem cells (ESCs) decreases markers of ESCs, including Sox2, Oct4, Nanog, and FGF4 (51), and increases the interaction between the lamina-associated domain and lamin B1 (64), implying that BAF

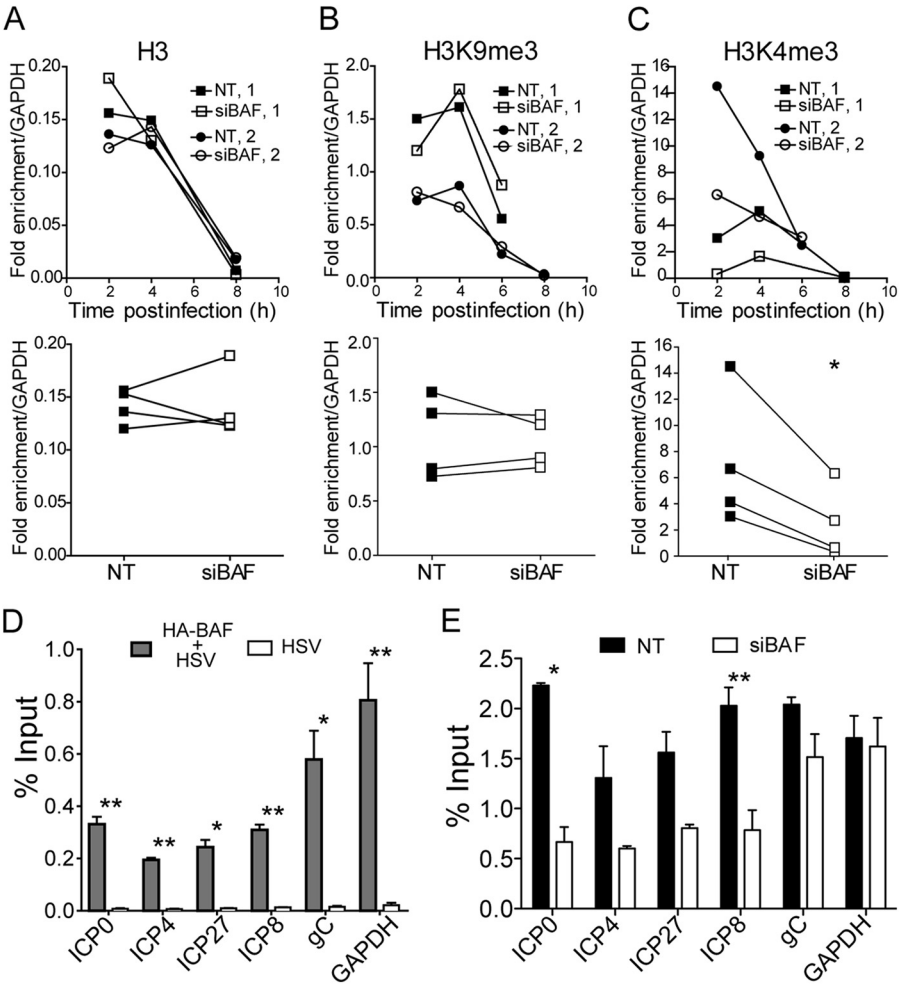

FIG 7 Effect of BAF depletion on viral chromatin. ChIP assays were performed to determine the levels of histone H3, the H3K9me3 heterochromatin mark, and the H3K4me3 euchromatin mark on the *ICP27* IE gene promoter. (A to C) HeLa cells transfected with NT control or BAF-specific siRNA were infected with HSV-1 at an MOI of 1 and fixed at the postinfection times indicated (top panels) or 2 hpi (bottom panels). Chromatin was prepared and immunoprecipitated with antibodies specific for H3 (A), H3K9me3 (B), and H3K4me3 (C), and the amounts of *ICP27* promoter sequences were determined by quantitative PCR. The top panels present two independent experiments, and the bottom panels present three or more independent experiments. Lines connect values of individual experiments. (D and E) ChIP assays were performed to determine the levels of BAF (D) and SETD1A (E) on viral gene promoters. HA-BAF was expressed transiently in HeLa cells that were then infected with HSV-1 at an MOI of 5. FLAG-SETD1A was expressed transiently in BAF-depleted HeLa (siBAF) or control (NT) cells that were then infected with HSV-1 at an MOI of 5. At 2 hpi, the cells were fixed and ChIP was performed with antibodies specific for HA or FLAG. The levels of individual HA-BAF- or FLAG-SETD1A-associated promoter sequences were determined by quantitative PCR. The histogram shows the mean values and standard deviations from three independent experiments. *, $P < 0.05$; **, $P \leq 0.01$ (two-tailed paired *t* test).

may also have positive functions in cellular gene expression rather than being limited to a repressive function. Although a recent study with murine cells showed that a mutant form of BAF could repress HSV replication (59), our results with human cells showed that WT BAF promotes HSV-1 lytic infection. The different results may be due to the difference in the host cells used, but it is more likely that the mutant BAF protein exerted a dominant negative effect on endogenous BAF. Consistent with this model, WT BAF did not inhibit HSV-1 replication in that study (59).

We examined the stages of the HSV life cycle at which BAF functions. Depletion of BAF reduces the levels of viral IE transcripts, but entry of the viral genome and accumulation of the tegument protein VP16 in the nucleus were not affected, implying that BAF plays a role after genome entry into the nucleus. Our ChIP assay results support a positive functional role for BAF in gene expression by serving as an epigenetic regulator. When we depleted BAF, we observed that the H3K4me3 euchromatin mark was decreased, but the heterochromatin mark H3K9me3 and total histone H3 were not significantly changed. Although overexpression of BAF has been reported to alter the global levels of histone modifications (60), our results argue that BAF contributes to modification of the H3K4me3 euchromatin mark specifically at viral promoters rather than by global changes in epigenetic profiles during HSV lytic infection. Nevertheless, we cannot exclude the possibility that other histone modifications and their regulating factors contribute to herpesvirus gene expression, and further studies are needed to identify other factors regulated by BAF for herpesvirus gene expression.

We found that the HSV ICP8 DNA-binding protein could interact with BAF. BAF accumulates in RCs in the nucleus, and

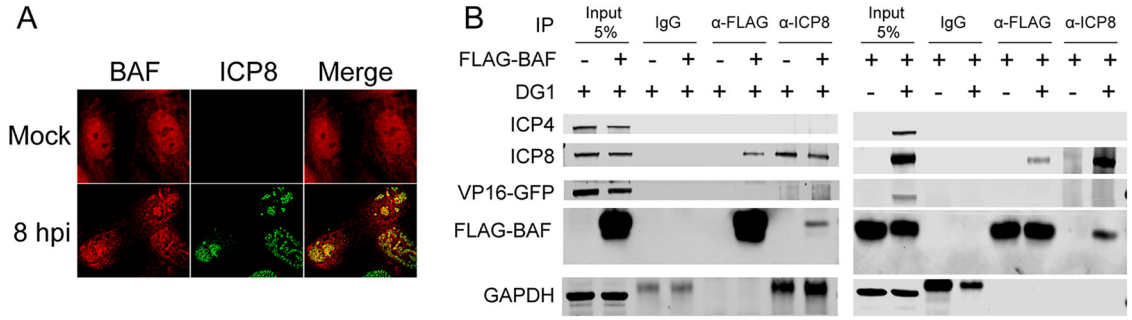

FIG 8 BAF localizes to viral RCs. (A) HFF cells were infected with HSV-1 at an MOI of 10, and an indirect immunofluorescence assay was performed with antibodies specific for BAF and ICP8 at 8 hpi. Images were deconvoluted by the inverse filter algorithm in the AxioVision 4.8 image acquisition software. (B) HeLa cell expressing FLAG-BAF were infected with HSV-1 DG1 at an MOI of 50, harvested at 4 hpi, and immunoprecipitated (IP) with antibodies specific for FLAG or ICP8. ICP4, ICP8, VP16-GFP, and FLAG-BAF were detected with antibodies specific for ICP4, ICP8, GFP, and FLAG.

coimmunoprecipitation results show an association of BAF with HSV ICP8. ICP8 binds to both single-stranded DNA and dsDNA (65, 66), and it is essential for viral genome replication (67–69). BAF has been proposed to promote cellular DNA replication. BAF localizes predominantly in the nucleus during S phase (43). BAF knockdown HeLa cells showed a 4-h extension of S phase compared to control cells (43), and HeLa cells overexpressing BAF showed significantly fewer cells in S phase (60), implying that BAF is required for optimal DNA replication. BAF has also been proposed to facilitate DNA damage repair on the basis of the association of BAF with multiple proteins involved in DNA damage repair (41). Therefore, the ICP8-BAF association raises the possibility of additional functions of BAF during viral replication.

Mechanism of BAF-mediated viral gene expression. Viruses target key regulatory mechanisms in their host cells, and the study of viral replication often identifies new cellular mechanisms. Thus, the functional recruitment of SETD1A methyltransferase onto viral promoters by BAF may define a new cellular mechanism (Fig. 9). We observed that SETD1A associated with all of the promoters tested at 2 hpi, but depletion of BAF reduced the association of the SETD1A methyltransferase and its corresponding histone modification, H3K4me3, with HSV IE and E gene promoters preferentially. These results argue that the association with the L (*gC*) gene promoter is not functional or regulated by a different mechanism(s). On the basis of these results, we hypothesize that BAF may need viral and/or cellular binding partners to achieve its gene specificity.

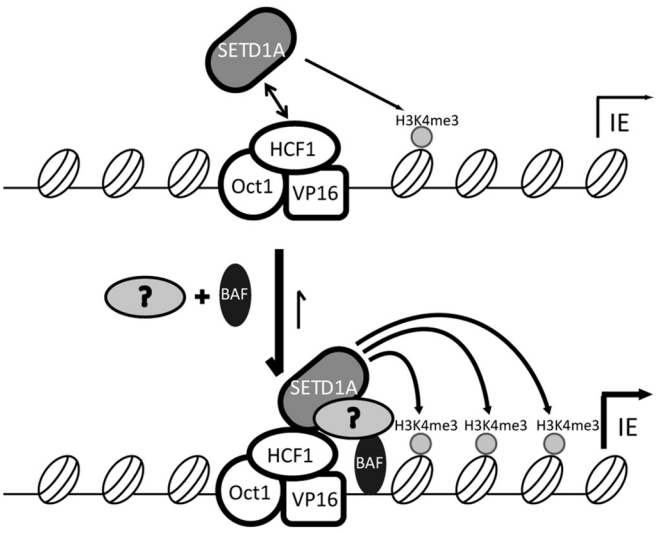

FIG 9 Model of the role of BAF in HSV gene transcription. SETD1A is recruited to the VP16 transactivator complex (VP16-Oct1-HCF1), and BAF (and unknown factors) stabilizes the association of SETD1A with the VP16 transactivator complex to enhance IE gene transcription.

HSV VP16 forms a transactivator complex with the cellular HCF-1 and Oct-1 proteins, and this complex specifically recognizes IE gene promoter sequences (ATGCTAATGARAT) (4–9). HCF-1 in the VP16 transactivator complex is a key factor that recruits various epigenetic modifiers, including SETD1A (9, 21), suggesting that BAF might associate with VP16 or HCF-1 to gain its specificity. However, we could not coimmunoprecipitate VP16 and BAF (70) or HCF-1 and BAF (results not shown), suggesting that the interactions are transient or that the mechanism of BAF and SETD1A recruitment on IE gene promoters might not be directly related to VP16 or HCF-1. VP16 reduces histone H3 and heterochromatin H3K9me3 levels on IE gene promoters (28, 71), but depletion of BAF did not change the level of either. In addition, depletion of BAF did not affect the association between VP16 and IE gene promoters, supporting the idea that BAF might not contribute to interaction of the VP16 transactivator complex. We cannot exclude the possibility of an association between BAF and the VP16 transactivator complex, but BAF might be involved in post-VP16 transactivator complex formation steps, including SETD1A recruitment to the VP16 transactivator complex and/or the stability of the transactivator complex-SETD1A association. It is also possible that BAF might affect the association of other transcription factors and cofactors, including Oct-1, Sp1, HCF-1, and GABP, with viral promoters, and further studies are needed to clarify this.

The functional specificity of BAF for viral genes likely involves cellular factors. Although our results showed that BAF associates broadly with viral and cellular DNA, the levels varied with different genes. More interestingly, the levels of SETD1A association are reduced only on IE and E viral gene promoters in BAF-depleted cells, implying that an additional factor or factors might coordinate with BAF to gain the specificity of SETD1A recruitment to these specific genes. Previous studies also support this idea, because BAF recruits gene-specific transcriptional and epigenetic regulators, including cone-rod homeobox (Crx), beta-catenin, germ cell-less (GCL), retinoblastoma (Rb), and SMAD, through

its binding partners, mostly LEM domain-containing inner nuclear membrane proteins (34, 39, 60, 72–76). Therefore, we propose a mechanism in which cellular binding partners, potentially LEM domain-containing proteins, might also be required for the epigenetic regulation mediated by BAF and SETD1A for viral gene expression (Fig. 9). We observed that SETD1A expressed by transfection associated with all viral promoters at 2 hpi, but the kinetics of SETD1A association with individual viral promoters has not been evaluated. Therefore, it is not clear whether the levels of the associations of SETD1A with viral promoters are saturated because of the overexpression of SETD1A. Kinetic studies of association of native SETD1A or stably expressing SETD1A with viral promoters by ChIP assay could clarify this.

Trimethylation of H3K4 can be induced by multiple lysine methyltransferases, including SETD1A, SETD1B, MLL1 to -4, ASH1L, SMYD1, SMYD3, and Meisetz (77). Although their individual target genes have not been fully identified, they seem to regulate distinct genes. The localization patterns of SETD1A and SETD1B do not overlap in the nucleus (78), and SETD1B overexpression cannot rescue the defect of proliferation induced by SETD1A depletion in mouse ESCs (79). MLL2 is required for the fertility of oocytes, but SETD1A is not (79, 80). Individual MLLs are required for different functions in stem cells (81). It is possible that these functional differences depend on their specific subunits in each complex. SETD1A-B and MLL1 to -4, which contain human COMPASS-like complexes, have core subunits in common, including WDR5, RBBP5, ASH2L, and DPY30 (82), but SETD1A/B complexes are associated with WDR82 and CFP1; MLL1/2 complexes are associated with MENIN, PC4, and PSIP1; and MLL3/4 complexes are associated with UTX, PTIP, PA1, and NCOA6 (81, 82). Interestingly, DPY30 was detected in BAF immunoprecipitates from HeLa cell lysates (41), providing a possible interaction for the model proposed in Fig. 9. SETD1A and MLL1 have been shown to regulate alphaherpesvirus replication (83), but the other COMPASS-like complexes have not been observed to play a role in herpesvirus replication. Further study is needed to understand the specificity and/or redundancy of functional contributions of other COMPASS-like complexes for herpesvirus replication and cellular gene regulation, and HSV can provide a useful tool to understand it.

In summary, we have provided evidence that BAF could serve as an epigenetic mediator for optimal HSV-1 gene transcription. This is the first demonstration that BAF can regulate H3K4me3 to promote gene expression. Additionally, our results suggest that BAF can specifically induce the association of SETD1A on certain gene promoters. On the basis of our results and previous studies, we hypothesize that BAF can serve as an epigenetic mediator in both repressing and activating cellular gene expression, depending on the binding partners and chromatin status. This study also supports the idea that HSV can be a useful model system to understand the epigenetic regulation of gene expression.

MATERIALS AND METHODS

Cells, viruses, and drug treatments. HeLa, HFF, and Vero cells were obtained from the American Type Culture Collection. U2OS and Vero cells were maintained in Dulbecco's modified Eagle medium (DMEM; Life Technologies and Corning) supplemented with 5% (vol/vol) bovine calf serum (BCS; Life Technologies), 5% (vol/vol) fetal bovine serum (FBS; Life Technologies), and 2 mM L-glutamine in 5% CO_2. HFF cells were maintained in DMEM supplemented with 10% (vol/vol) FBS. WT HSV-1 KOS (84) and VP16-GFP-tagged HSV-1 DG1 (85) were grown in and their titers were determined on Vero cells. Infections were conducted with virus diluted in phosphate-buffered saline (PBS) containing 0.1% glucose (wt/vol) and 0.1% BCS (vol/vol) for 1 h with shaking at 37°C; this was followed by a medium change to DMEM–1% BCS and incubation at 37°C until the times indicated for harvesting.

Plasmid construction. HA-tagged *BANF1* coding sequences were subcloned into pLPCX (Clontech) to construct a pLPCX-HA-BAF plasmid. *HA-BANF1* was amplified by PCR amplification with primers 5′ C GTAAGCTTTCACAAGAAGGCGTCGCACCAC 3′ and 5′ ACCAAGCT TGCCACCATGGATTACAAGGATGACGATGACAAGCTGATGACAA CCTCCCAAAAGC 3′ (Integrated DNA Technologies) from the pFLAG-BAF (44) plasmid (a gift from Katherine L. Wilson) with High Fidelity PCR Master (Roche) according to the manufacturer's protocol. The PCR product was digested with HindIII (New England Biolabs [NEB]) and purified with the QIAquick PCR Purification kit (Qiagen). The pLPCX plasmid was digested with HindIII (NEB), treated with shrimp alkaline phosphatase (NEB), and purified from low-melting-point agarose gel with the QIAquick gel extraction kit (Qiagen). The digested PCR fragment was ligated into pLPCX with T4 DNA ligase (Life Technologies), and the ligation mixture was transformed to NEB 5-alpha competent *Escherichia coli* cells (NEB).

siRNA-mediated BAF depletion. ON-TARGETplus SMARTpool and its individual siRNAs targeting *BANF1* (L-011536-01, J-011536-9, J-011536-10, J-011536-11, and J-011536-12) and a Nontargeting Control Pool (D-001810-10) were obtained from Thermo Fisher Scientific. The double-stranded siRNAs were transfected into HeLa or HFF cells at 5 or 50 nM with the HiPerFect reagent (Qiagen) or Dharmafect II, respectively, according to the manufacturer's protocol. After 3 days, the cells were passaged and a second transfection to HeLa or HFF cells at a final concentration of 5 or 50 nM, respectively, was performed. At 3 days after the second siRNA transfection, cells were counted and infected with HSV-1 at the MOIs indicated.

SDS-PAGE and immunoblotting. For analysis of total proteins by immunoblotting, cells were lysed in lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EGTA) containing 1× Complete protease cocktail inhibitors (Roche) and 1× NuPAGE sample buffer (Life Technologies) as previously described (86). The proteins in the lysates were resolved in NuPAGE 4 to 12% bis-Tris gels (Life Technologies) and then transferred to a polyvinylidene difluoride (PerkinElmer Life Sciences) or nitrocellulose membrane (Bio-Rad). The membrane was blocked in 5% skim milk in Tris-buffered saline with 0.1% Tween 20 or Odyssey blocking buffer (LI-COR). The membrane was then incubated with antibodies specific for HSV ICP8 (1:5,000, rabbit serum 3-83 [87]), HSV ICP4 (1:4,000, mouse monoclonal antibody [MAb] 58S [88]), ICP27 (1:5,000, MAb; Eastcoast Bio), gD (1:5,000, MAb; Eastcoast Bio), BAF (1:2,000, rabbit serum; Millipore), GFP (1:2,000, rabbit serum; Abcam), lamin B1 (1:10,000, rabbit serum; Abcam), GAPDH (1:10,000, MAb; Abcam), and FLAG M2 (1:2,000, MAb; Sigma). The membrane was incubated with secondary antibodies (goat anti-mouse IgG-horseradish peroxidase [HRP], goat anti-rabbit IgG-HRP [Santa Cruz Biotechnology], or IRDye 680RD and IRDye 800 [LI-COR]) for 45 min. The HRP signal was detected with chemiluminescence reagents (Luminata Western substrate; Millipore) and X-ray film (HyBlot ES; Denville Scientific). Near-infrared fluorescence was detected with Odyssey (LI-COR). Protein expression levels were quantified with ImageJ or Image Studio Lite (LI-COR).

Quantification of RNA levels by RT and real-time PCR. Quantification of RNA was performed as described previously (86). In brief, total RNA was purified with the RNeasy minikit (Qiagen), 1 μg of the total RNA was treated with DNase I (Ambion) at 37°C for 1 h, and half of the DNase I-treated RNA was reverse transcribed with the high-capacity cDNA RT kit (Applied Biosystems). Relative amounts of specific cDNAs were quantified by using their specific primers with the SYBR green PCR master mix reagent (Applied Biosystems) and the StepOnePlus real-time PCR system (Life Technologies). The primers (Integrated DNA Technol-

ogies) used in this study were as follows: for ChIP assays, ICP0 (5′ CGCT CAATGAACCCGCATT 3′ and 5′ CGCCTTCCCGAAGAAACTCA 3′ [89]), ICP4 (5′ TAGCATGCGGAACGGAAGC 3′ and 5′ CGCATGGCA TCTCATTACCG 3′ [90]), ICP8 (5′ TGCTTACGGTCAGGTGCTCCG 3′ and 5′ CCACGCCCACCGGCTGATGAC 3′ [61]), ICP27 (5′ CCGCCG GCCTGGATGTGACG 3′ and 5′ CGTGGTGGCCGGGGTGGTGCTC 3′ [91]), gC (5′ CCGACCGCCCGCCCGTTGAC 3′ and 5′ ACGCTTCGGG GCCTCTTCTTCTCC 3′ [61]), GAPDH (5′ TTCGACAGTCAGCCGCA TCTTCTT 3′ and 5′ CAGGCGCCCAATACGACCAAATC 3′ [61]) and GAPDH promoter (5′ TACTAGCGGTTTTACGGGCG 3′ and 5′ TCGA ACAGGAGGAGCAGAGAGCGA 3′); for transcripts and vDNA, ICP4 (5′ GCGTCGTCGAGGTCGT 3′ and 5′ CGCGGAGACGGAGGAG 3′ [92]), ICP27 (5′ GCATCCTTCGTGTTTGTCATT 3′ and 5′ GCATCTT CTCTCCGACCCCG 3′ [92]), ICP8 (5′ GTCGTTACCGAGGGCTTCAA 3′ and 5′ GTTACCTTGTCCGAGCCTCC 3′), BANF1 (5′ TGGCCAGT TTCTGGTGC 3′ and 5′ CGAAGGCATCCGAAGCAG 3′), and 18S rRNA (5′ GTAACCCGTTGAACCCCATT 3′ and 5′ CCATCCAATCGGTAGT AGCG 3′).

Cell fractionation. For immunoblotting, HFF cells were infected with HSV-1 DG1 at an MOI of 100 and harvested at 3 hpi. Cell fractionation was performed with the NE-PER Nuclear Protein Extraction kit (Pierce) according to the manufacturer's protocol. The cytoplasmic and nuclear fractions were subjected to SDS-PAGE and immunoblotting. For viral DNA quantification, BAF-depleted HFF cells were infected with HSV-1 at an MOI of 10 and harvested at 2 hpi and nuclei were isolated with the NE-PER Nuclear Protein Extraction kit (Pierce). Viral DNA was purified from separated nuclei with the DNeasy Blood and Tissue kit (Qiagen) and quantified with the StepOnePlus real-time PCR system (Life Technologies).

Immunofluorescence microscopy. HeLa or HFF cells were seeded at 1×10^5 or 5×10^4/15.6-mm well on glass coverslips at 24 h prior to infection. At the postinfection times indicated, cells were fixed with 3.7% paraformaldehyde in PBS for 10 min and permeabilized with 0.1% Triton X-100 in PBS for 10 min (28). Antibodies specific for histone H3K9me3 (Abcam) and HSV-1 ICP4 58S (88) and HSV-1 ICP8 mouse MAb 39S (87) were incubated for 1 h at room temperature. Secondary antibodies conjugated to the dyes Alexa 594 and Alexa 488 were subsequently used for 1 h at room temperature. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Life Technologies), and ProLong Gold Antifade mounting reagent (Life Technologies) was used to mount coverslips. Wide-field images of cells were acquired on a Zeiss Axioplan 2 microscope with a Plan Apochromat 1.4 numerical aperture 63× objective lens, a Photometrics CoolSNAP HQ2 charge-coupled device camera, and the Zeiss AxioVision 4.8 image acquisition software.

Immunoprecipitation. HeLa cells (1.5×10^6) were seeded into 100-mm dishes, transfected with 2 μg of either pFLAG-BAF (a gift from Katherine L. Wilson) or the empty-vector control, and at 24 to 48 h post-transfection, the cells were infected with HSV-1 DG1 at an MOI of 50. At 4 hpi, cells were harvested and lysed with NP-40 lysis buffer (0.5% NP-40, 10% glycerol, 50 mM Tris [pH 7.5], 150 mM NaCl, 50 mM NaF, 1× Complete protease cocktail inhibitors [Roche]) on ice for 20 min. Supernatant samples were recovered after centrifugation at 1,000 × *g* at 4°C for 5 min. Pellets were resuspended in NP-40 lysis buffer again and sonicated with Bioruptor (Diangenode) for 20 s at maximum amplitude. The saved supernatant and sonicated samples were combined and spun at maximum speed in a microcentrifuge at 4°C to separate the supernatant and pellet. The supernatant lysates were subjected to immunoprecipitation with an antibody specific for FLAG (M2, Sigma) or ICP8 (3–83, 87) or with control normal rabbit IgG (Millipore). After four or five washes with NP-40 lysis buffer, the beads were boiled in Laemmli sample buffer and the proteins were resolved by SDS-PAGE.

ChIP assays. ChIP assays were performed as described previously, with modifications (28, 93). HeLa cells were seeded at 3×10^6/100-mm dish at 24 h prior to infection. Cells were infected at an MOI of 1 or 10 with WT HSV-1 or HSV-1 DG1, respectively. For FLAG-SETD1A or HA-BAF ChIP assay, HeLa cells transfected with pcDNA3 FLAG-SETD1A (a gift from David Skalnik and Jeong-Heon Lee) or pLPCX-HA-BAF were infected with WT HSV-1 at an MOI of 5. At the postinfection times indicated, the cells were fixed with 1% formaldehyde for 10 min (ChIP for histones) or 20 min (others) at room temperature, glycine at 125 mM was added to quench the formaldehyde, and the mixture was incubated for 3 min. The cells were washed twice with cold PBS, scraped into cold PBS supplemented with 1× Complete protease cocktail inhibitors (Roche), and pelleted for 5 min at 1,000 × *g*. The cells were frozen in liquid nitrogen and kept at −80°C. The cells were thawed on ice, lysed in cold lysis buffer I (50 mM HEPES-KOH [pH 7.5], 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, 140 mM NaCl, 1 mM EDTA, 1× Complete protease cocktail inhibitors) on ice for 10 min, and collected by centrifugation for 10 min at 1,000 × *g*. Pellets were resuspended in cold lysis buffer II (10 mM Tris-HCl [pH 8.0], 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1× Complete protease cocktail inhibitors), rotated for 10 min at 4°C, and then spun for 10 min at 1,000 × *g*. Pelleted nuclei were resuspended in lysis buffer III (10 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.1% sodium deoxycholate, 0.5% *N*-lauroylsarcosine, 1 mM EDTA, 0.5 mM EGTA, 1× Complete protease cocktail inhibitors). The pelleted nuclei were sonicated with a Bioruptor 200 (Diagenode) for multiple cycles of 30 s on and 30 s off at the maximum power until the majority of the chromatin fragments were less than 1 kbp. The chromatin was clarified by centrifugation and diluted 10-fold in ChIP dilution buffer (150 mM NaCl, 10 mM sodium phosphate, 2 mM EDTA, 1.1% Triton X-100, 0.1% SDS, 1× Complete protease cocktail inhibitors). Aliquots of chromatins (20 to 50 μg) were incubated overnight with 2.5 μg of anti-histone H3 IgG (Abcam ab1791), anti-histone H3K9me3 IgG (Abcam ab8898), anti-histone H3K4me3 IgG (Abcam ab8580), or anti-GFP (Abcam ab290 and Clontech JL-8) antibody or rabbit IgG as a negative control (Millipore). For the FLAG-SETD1A ChIP assay, we used 2.5 μg of anti-FLAG M2 IgG (Sigma F3165) or mouse monoclonal IgG as a negative control (Millipore). Prior to the addition of each antibody, 5% of each sample was saved to determine the input value. Magna ChIP protein A/G (20 μl for anti-FLAG) or A (20 μl for other antibodies from rabbits) magnetic beads (Millipore) were added to each sample. For the HA-BAF ChIP assay, we added anti-HA magnetic beads (Pierce 88836) to lysates of pLPCX-HA-BAF or pLPCX plasmid-transfected cells. The samples were incubated for 1 h at 4°C and washed four times with ChIP dilution buffer, twice with lithium chloride wash buffer (50 mM HEPES [pH 7.5], 250 mM lithium chloride, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, 0.7% sodium deoxycholate, 1× Complete protease cocktail inhibitors), and twice with Tris-EDTA (TE; 10 mM Tris-HCl [pH 8.0], 1 mM EDTA) buffer. DNAs were eluted twice with 90 μl of elution buffer (1% SDS, 100 mM sodium bicarbonate) to 65°C for 10 min. TE buffer (pH 8.0), NaCl (to a concentration of 200 mM), and 1 μg RNase A (Ambion) were added, and the cross-links were reversed at 65°C overnight. The samples were treated with proteinase K (Roche) for 1 h at 45°C, and the DNAs were purified with the QIAquick PCR purification kit (Qiagen).

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REFERENCES

1. **Muggeridge MI, Fraser NW.** 1986. Chromosomal organization of the herpes simplex virus genome during acute infection of the mouse central nervous system. J Virol **59:**764–767.
2. **Leinbach SS, Summers WC.** 1980. The structure of herpes simplex virus

type 1 DNA as probed by micrococcal nuclease digestion. J Gen Virol **51:**45–59. http://dx.doi.org/10.1099/0022-1317-51-1-45.
3. **Kent JR, Zeng PY, Atanasiu D, Gardner J, Fraser NW, Berger SL.** 2004. During lytic infection herpes simplex virus type 1 is associated with histones bearing modifications that correlate with active transcription. J Virol **78:**10178–10186. http://dx.doi.org/10.1128/JVI.78.18.10178-10186.2004.
4. **Mackem S, Roizman B.** 1982. Structural features of the herpes simplex virus alpha gene 4, 0, and 27 promoter-regulatory sequences which confer alpha regulation on chimeric thymidine kinase genes. J Virol **44:**939–949.
5. **Kristie TM, Roizman B.** 1984. Separation of sequences defining basal expression from those conferring alpha gene recognition within the regulatory domains of herpes simplex virus 1 alpha genes. Proc Natl Acad Sci U S A **81:**4065–4069. http://dx.doi.org/10.1073/pnas.81.13.4065.
6. **Gerster T, Roeder RG.** 1988. A herpesvirus trans-activating protein interacts with transcription factor OTF-1 and other cellular proteins. Proc Natl Acad Sci U S A **85:**6347–6351. http://dx.doi.org/10.1073/pnas.85.17.6347.
7. **Kristie TM, LeBowitz JH, Sharp PA.** 1989. The octamer-binding proteins form multi-protein–DNA complexes with the HSV alpha TIF regulatory protein. EMBO J **8:**4229–4238.
8. **Greaves RF, O'Hare P.** 1990. Structural requirements in the herpes simplex virus type 1 transactivator Vmw65 for interaction with the cellular octamer-binding protein and target TAATGARAT sequences. J Virol **64:**2716–2724.
9. **Vogel JL, Kristie TM.** 2013. The dynamics of HCF-1 modulation of herpes simplex virus chromatin during initiation of infection. Viruses **5:**1272–1291. http://dx.doi.org/10.3390/v5051272.
10. **Uesugi M, Nyanguile O, Lu H, Levine AJ, Verdine GL.** 1997. Induced alpha helix in the VP16 activation domain upon binding to a human TAF. Science **277:**1310–1313. http://dx.doi.org/10.1126/science.277.5330.1310.
11. **Kobayashi N, Boyer TG, Berk AJ.** 1995. A class of activation domains interacts directly with TFIIA and stimulates TFIIA-TFIID-promoter complex assembly. Mol Cell Biol **15:**6465–6473.
12. **Lin YS, Ha I, Maldonado E, Reinberg D, Green MR.** 1991. Binding of general transcription factor TFIIB to an acidic activating region. Nature **353:**569–571. http://dx.doi.org/10.1038/353569a0.
13. **Stringer KF, Ingles CJ, Greenblatt J.** 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature **345:**783–786. http://dx.doi.org/10.1038/345783a0.
14. **Ingles CJ, Shales M, Cress WD, Triezenberg SJ, Greenblatt J.** 1991. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature **351:**588–590. http://dx.doi.org/10.1038/351588a0.
15. **Ito M, Yuan CX, Malik S, Gu W, Fondell JD, Yamamura S, Fu ZY, Zhang X, Qin J, Roeder RG.** 1999. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Mol Cell **3:**361–370. http://dx.doi.org/10.1016/S1097-2765(00)80463-3.
16. **Park JM, Kim HS, Han SJ, Hwang MS, Lee YC, Kim YJ.** 2000. In vivo requirement of activator-specific binding targets of mediator. Mol Cell Biol **20:**8709–8719. http://dx.doi.org/10.1128/MCB.20.23.8709-8719.2000.
17. **Mittler G, Stühler T, Santolin L, Uhlmann T, Kremmer E, Lottspeich F, Berti L, Meisterernst M.** 2003. A novel docking site on mediator is critical for activation by VP16 in mammalian cells. EMBO J **22:**6494–6504. http://dx.doi.org/10.1093/emboj/cdg619.
18. **Gunther M, Laithier M, Brison O.** 2000. A set of proteins interacting with transcription factor Sp1 identified in a two-hybrid screening. Mol Cell Biochem **210:**131–142. http://dx.doi.org/10.1023/A:1007177623283.
19. **Vogel JL, Kristie TM.** 2000. The novel coactivator C1 (HCF) coordinates multiprotein enhancer formation and mediates transcription activation by GABP. EMBO J **19:**683–690. http://dx.doi.org/10.1093/emboj/19.4.683.
20. **Vogel JL, Kristie TM.** 2006. Site-specific proteolysis of the transcriptional coactivator HCF-1 can regulate its interaction with protein cofactors. Proc Natl Acad Sci U S A **103:**6817–6822. http://dx.doi.org/10.1073/pnas.0602109103.
21. **Wysocka J, Myers MP, Laherty CD, Eisenman RN, Herr W.** 2003. Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1. Genes Dev **17:**896–911. http://dx.doi.org/10.1101/gad.252103.
22. **Liang Y, Vogel JL, Narayanan A, Peng H, Kristie TM.** 2009. Inhibition of the histone demethylase LSD1 blocks alpha-herpesvirus lytic replication and reactivation from latency. Nat Med **15:**1312–1317. http://dx.doi.org/10.1038/nm.2051.
23. **Liang Y, Vogel JL, Arbuckle JH, Rai G, Jadhav A, Simeonov A, Maloney DJ, Kristie TM.** 2013. Targeting the JMJD2 histone demethylases to epigenetically control herpesvirus infection and reactivation from latency. Sci Transl Med **5:**167ra165. http://dx.doi.org/10.1126/scitranslmed.3005145.
24. **Knipe DM, Lieberman PM, Jung JU, McBride AA, Morris KV, Ott M, Margolis D, Nieto A, Nevels M, Parks RJ, Kristie TM.** 2013. Snapshots: chromatin control of viral infection. Virology **435:**141–156. http://dx.doi.org/10.1016/j.virol.2012.09.023.
25. **de Bruyn Kops A, Knipe DM.** 1988. Formation of DNA replication structures in herpes virus-infected cells requires a viral DNA binding protein. Cell **55:**857–868. http://dx.doi.org/10.1016/0092-8674(88)90141-9.
26. **Everett RD, Murray J.** 2005. ND10 components relocate to sites associated with herpes simplex virus type 1 nucleoprotein complexes during virus infection. J Virol **79:**5078–5089. http://dx.doi.org/10.1128/JVI.79.8.5078-5089.2005.
27. **Silva L, Cliffe A, Chang L, Knipe DM.** 2008. Role for A-type lamins in herpesviral DNA targeting and heterochromatin modulation. PLoS Pathog **4:**e1000071. http://dx.doi.org/10.1371/journal.ppat.1000071.
28. **Silva L, Oh HS, Chang L, Yan Z, Triezenberg SJ, Knipe DM.** 2012. Roles of the nuclear lamina in stable nuclear association and assembly of a herpesviral transactivator complex on viral immediate-early genes. mBio **3:**e00300-00311. http://dx.doi.org/10.1128/mBio.00300-11.
29. **Mou F, Wills EG, Park R, Baines JD.** 2008. Effects of lamin A/C, lamin B1, and viral US3 kinase activity on viral infectivity, virion egress, and the targeting of herpes simplex virus U(L)34-encoded protein to the inner nuclear membrane. J Virol **82:**8094–8104. http://dx.doi.org/10.1128/JVI.00874-08.
30. **Zheng R, Ghirlando R, Lee MS, Mizuuchi K, Krause M, Craigie R.** 2000. Barrier-to-autointegration factor (BAF) bridges DNA in a discrete, higher-order nucleoprotein complex. Proc Natl Acad Sci U S A **97:**8997–9002. http://dx.doi.org/10.1073/pnas.150240197.
31. **Coutinho HD, Falcão-Silva VS, Gonçalves GF, da Nóbrega RB.** 2009. Molecular ageing in progeroid syndromes: Hutchinson-Gilford progeria syndrome as a model. Immun Ageing **6:**4. http://dx.doi.org/10.1186/1742-4933-6-4.
32. **Cai M, Huang Y, Zheng R, Wei SQ, Ghirlando R, Lee MS, Craigie R, Gronenborn AM, Clore GM. 1998.** Solution structure of the cellular factor BAF responsible for protecting retroviral DNA from autointegration. Nat Struct Biol **5:**903–909. http://dx.doi.org/10.1038/2345.
33. **Segura-Totten M, Wilson KL.** 2004. BAF: roles in chromatin, nuclear structure and retrovirus integration. Trends Cell Biol **14:**261–266. http://dx.doi.org/10.1016/j.tcb.2004.03.004.
34. **Margalit A, Brachner A, Gotzmann J, Foisner R, Gruenbaum Y.** 2007. Barrier-to-autointegration factor—a BAFfling little protein. Trends Cell Biol **17:**202–208. http://dx.doi.org/10.1016/j.tcb.2007.02.004.
35. **Umland TC, Wei SQ, Craigie R, Davies DR.** 2000. Structural basis of DNA bridging by barrier-to-autointegration factor. Biochemistry **39:**9130–9138. http://dx.doi.org/10.1021/bi000572w.
36. **Skoko D, Li M, Huang Y, Mizuuchi M, Cai M, Bradley CM, Pease PJ, Xiao B, Marko JF, Craigie R, Mizuuchi K.** 2009. Barrier-to-autointegration factor (BAF) condenses DNA by looping. Proc Natl Acad Sci U S A **106:**16610–16615. http://dx.doi.org/10.1073/pnas.0909077106.
37. **Bradley CM, Ronning DR, Ghirlando R, Craigie R, Dyda F.** 2005. Structural basis for DNA bridging by barrier-to-autointegration factor. Nat Struct Mol Biol **12:**935–936. http://dx.doi.org/10.1038/nsmb989.
38. **Furukawa K.** 1999. LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction. J Cell Sci **112:**2485–2492.
39. **Wang X, Xu S, Rivolta C, Li LY, Peng GH, Swain PK, Sung CH, Swaroop A, Berson EL, Dryja TP, Chen S.** 2002. Barrier to autointegration factor interacts with the cone-rod homeobox and represses its transactivation function. J Biol Chem **277:**43288–43300. http://dx.doi.org/10.1074/jbc.M207952200.
40. **Montes de Oca R, Lee KK, Wilson KL.** 2005. Binding of barrier to autointegration factor (BAF) to histone H3 and selected linker histones including H1.1. J Biol Chem **280:**42252–42262. http://dx.doi.org/10.1074/jbc.M509917200.
41. **Montes de Oca R, Shoemaker CJ, Gucek M, Cole RN, Wilson KL.** 2009. Barrier-to-autointegration factor proteome reveals chromatin-regulatory

partners. PLoS One **4:**e7050. http://dx.doi.org/10.1371/journal.pone.0007050.
42. **Haraguchi T, Koujin T, Segura-Totten M, Lee KK, Matsuoka Y, Yoneda Y, Wilson KL, Hiraoka Y.** 2001. BAF is required for emerin assembly into the reforming nuclear envelope. J Cell Sci **114:**4575–4585.
43. **Haraguchi T, Koujin T, Osakada H, Kojidani T, Mori C, Masuda H, Hiraoka Y.** 2007. Nuclear localization of barrier-to-autointegration factor is correlated with progression of S phase in human cells. J Cell Sci **120:**1967–1977. http://dx.doi.org/10.1242/jcs.03461.
44. **Nichols RJ, Wiebe MS, Traktman P.** 2006. The vaccinia-related kinases phosphorylate the N' terminus of BAF, regulating its interaction with DNA and its retention in the nucleus. Mol Biol Cell **17:**2451–2464. http://dx.doi.org/10.1091/mbc.E05-12-1179.
45. **Gorjánácz M, Klerkx EP, Galy V, Santarella R, López-Iglesias C, Askjaer P, Mattaj IW.** 2007. Caenorhabditis elegans BAF-1 and its kinase VRK-1 participate directly in post-mitotic nuclear envelope assembly. EMBO J **26:**132–143. http://dx.doi.org/10.1038/sj.emboj.7601470.
46. **Asencio C, Davidson IF, Santarella-Mellwig R, Ly-Hartig TB, Mall M, Wallenfang MR, Mattaj IW, Gorjánácz M.** 2012. Coordination of kinase and phosphatase activities by Lem4 enables nuclear envelope reassembly during mitosis. Cell **150:**122–135. http://dx.doi.org/10.1016/j.cell.2012.04.043.
47. **Zhuang X, Semenova E, Maric D, Craigie R.** 2014. Dephosphorylation of barrier-to-autointegration factor by protein phosphatase 4 and its role in cell mitosis. J Biol Chem **289:**1119–1127. http://dx.doi.org/10.1074/jbc.M113.492777.
48. **Molitor TP, Traktman P.** 2014. Depletion of the protein kinase VRK1 disrupts nuclear envelope morphology and leads to BAF retention on mitotic chromosomes. Mol Biol Cell **25:**891–903. http://dx.doi.org/10.1091/mbc.E13-10-0603.
49. **Margalit A, Segura-Totten M, Gruenbaum Y, Wilson KL.** 2005. Barrier-to-autointegration factor is required to segregate and enclose chromosomes within the nuclear envelope and assemble the nuclear lamina. Proc Natl Acad Sci U S A **102:**3290–3295. http://dx.doi.org/10.1073/pnas.0408364102.
50. **Furukawa K, Sugiyama S, Osouda S, Goto H, Inagaki M, Horigome T, Omata S, McConnell M, Fisher PA, Nishida Y.** 2003. Barrier-to-autointegration factor plays crucial roles in cell cycle progression and nuclear organization in drosophila. J Cell Sci **116:**3811–3823. http://dx.doi.org/10.1242/jcs.00682.
51. **Cox JL, Mallanna SK, Ormsbee BD, Desler M, Wiebe MS, Rizzino A.** 2011. Banf1 is required to maintain the self-renewal of both mouse and human embryonic stem cells. J Cell Sci **124:**2654–2665. http://dx.doi.org/10.1242/jcs.083238.
52. **Cabanillas R, Cadiñanos J, Villameytide JA, Pérez M, Longo J, Richard JM, Alvarez R, Durán NS, Illán R, González DJ, López-Otín C.** 2011. Nestor-Guillermo progeria syndrome: a novel premature aging condition with early onset and chronic development caused by BANF1 mutations. Am J Med Genet A **155A:**2617–2625. http://dx.doi.org/10.1002/ajmg.a.34249.
53. **Puente XS, Quesada V, Osorio FG, Cabanillas R, Cadiñanos J, Fraile JM, Ordóñez GR, Puente DA, Gutiérrez-Fernández A, Fanjul-Fernández M, Lévy N, Freije JM, López-Otín C.** 2011. Exome sequencing and functional analysis identifies BANF1 mutation as the cause of a hereditary progeroid syndrome. Am J Hum Genet **88:**650–656. http://dx.doi.org/10.1016/j.ajhg.2011.04.010.
54. **Dechat T, Pfleghaar K, Sengupta K, Shimi T, Shumaker DK, Solimando L, Goldman RD.** 2008. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev **22:**832–853. http://dx.doi.org/10.1101/gad.1652708.
55. **Chen H, Engelman A.** 1998. The barrier-to-autointegration protein is a host factor for HIV type 1 integration. Proc Natl Acad Sci U S A **95:**15270–15274. http://dx.doi.org/10.1073/pnas.95.26.15270.
56. **Lee MS, Craigie R.** 1998. A previously unidentified host protein protects retroviral DNA from autointegration. Proc Natl Acad Sci U S A **95:**1528–1533. http://dx.doi.org/10.1073/pnas.95.4.1528.
57. **Lin CW, Engelman A.** 2003. The barrier-to-autointegration factor is a component of functional human immunodeficiency virus type 1 preintegration complexes. J Virol **77:**5030–5036. http://dx.doi.org/10.1128/JVI.77.8.5030-5036.2003.
58. **Wiebe MS, Traktman P.** 2007. Poxviral B1 kinase overcomes barrier to autointegration factor, a host defense against virus replication. Cell Host Microbe **1:**187–197. http://dx.doi.org/10.1016/j.chom.2007.03.007.
59. **Jamin A, Thunuguntla P, Wicklund A, Jones C, Wiebe MS.** 2014. Barrier to Autointegration factor becomes dephosphorylated during HSV-1 infection and can act as a host defense by impairing viral DNA replication and gene expression. PLoS One **9:**e100511. http://dx.doi.org/10.1371/journal.pone.0100511.
60. **Montes de Oca R, Andreassen PR, Wilson KL.** 2011. Barrier-to-autointegration factor influences specific histone modifications. Nucleus **2:**580–590. http://dx.doi.org/10.4161/nucl.2.6.17960.
61. **Cliffe AR, Knipe DM.** 2008. Herpes simplex virus ICP0 promotes both histone removal and acetylation on viral DNA during lytic infection. J Virol **82:**12030–12038. http://dx.doi.org/10.1128/JVI.01575-08.
62. **Huang J, Kent JR, Placek B, Whelan KA, Hollow CM, Zeng PY, Fraser NW, Berger SL.** 2006. Trimethylation of histone H3 lysine 4 by Set1 in the lytic infection of human herpes simplex virus 1. J Virol **80:**5740–5746. http://dx.doi.org/10.1128/JVI.00169-06.
63. **Ibrahim N, Wicklund A, Wiebe MS.** 2011. Molecular characterization of the host defense activity of the barrier to autointegration factor against vaccinia virus. J Virol **85:**11588–11600. http://dx.doi.org/10.1128/JVI.00641-11.
64. **Kind J, van Steensel B.** 2014. Stochastic genome-nuclear lamina interactions: modulating roles of lamin A and BAF. Nucleus **5:**124–130. http://dx.doi.org/10.4161/nucl.28825.
65. **Ruyechan WT.** 1983. The major herpes simplex virus DNA-binding protein holds single-stranded DNA in an extended configuration. J Virol **46:**661–666.
66. **Lee CK, Knipe DM.** 1985. An immunoassay for the study of DNA-binding activities of herpes simplex virus protein ICP8. J Virol **54:**731–738.
67. **Conley AJ, Knipe DM, Jones PC, Roizman B.** 1981. Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of gamma polypeptides. J Virol **37:**191–206.
68. **Wu CA, Nelson NJ, McGeoch DJ, Challberg MD.** 1988. Identification of herpes simplex virus type 1 genes required for origin-dependent DNA synthesis. J Virol **62:**435–443.
69. **Boehmer PE, Lehman IR.** 1993. Herpes simplex virus type 1 ICP8: helix-destabilizing properties. J Virol **67:**711–715.
70. **Oh HS, Knipe DM.** 23 March 2015. Proteomic analysis of the herpes simplex virus 1 virion protein 16 transactivator protein in infected cells. Proteomics http://dx.doi.org/10.1002/pmic.201500020.
71. **Hancock MH, Cliffe AR, Knipe DM, Smiley JR.** 2010. Herpes simplex virus VP16, but not ICP0, is required to reduce histone occupancy and enhance histone acetylation on viral genomes in U2OS osteosarcoma cells. J Virol **84:**1366–1375. http://dx.doi.org/10.1128/JVI.01727-09.
72. **Nili E, Cojocaru GS, Kalma Y, Ginsberg D, Copeland NG, Gilbert DJ, Jenkins NA, Berger R, Shaklai S, Amariglio N, Brok-Simoni F, Simon AJ, Rechavi G.** 2001. Nuclear membrane protein LAP2beta mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less). J Cell Sci **114:**3297–3307. http://dx.doi.org/10.1083/jcb.200511149.
73. **Dorner D, Vlcek S, Foeger N, Gajewski A, Makolm C, Gotzmann J, Hutchison CJ, Foisner R.** 2006. Lamina-associated polypeptide 2alpha regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway. J Cell Biol **173:**83–93. http://dx.doi.org/10.1083/jcb.200511149.
74. **Markiewicz E, Tilgner K, Barker N, van de Wetering M, Clevers H, Dorobek M, Hausmanowa-Petrusewicz I, Ramaekers FC, Broers JL, Blankesteijn WM, Salpingidou G, Wilson RG, Ellis JA, Hutchison CJ.** 2006. The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. EMBO J **25:**3275–3285. http://dx.doi.org/10.1038/sj.emboj.7601230.
75. **Holaska JM, Lee KK, Kowalski AK, Wilson KL.** 2003. Transcriptional repressor germ cell-less (GCL) and barrier to autointegration factor (BAF) compete for binding to emerin in vitro. J Biol Chem **278:**6969–6975. http://dx.doi.org/10.1074/jbc.M208811200.
76. **Huang Y, Cai M, Clore GM, Craigie R.** 2011. No interaction of barrier-to-autointegration factor (BAF) with HIV-1 MA, cone-rod homeobox (Crx) or MAN1-C in absence of DNA. PLoS One **6:**e25123. http://dx.doi.org/10.1371/journal.pone.0025123.
77. **Lanouette S, Mongeon V, Figeys D, Couture JF.** 2014. The functional diversity of protein lysine methylation. Mol Syst Biol **10:**724. http://dx.doi.org/10.1002/msb.134974.
78. **Lee JH, Tate CM, You JS, Skalnik DG.** 2007. Identification and characterization of the human Set1B histone H3-Lys4 methyltransferase com-

plex. J Biol Chem **282:**13419–13428. http://dx.doi.org/10.1074/jbc.M609809200.
79. **Bledau AS, Schmidt K, Neumann K, Hill U, Ciotta G, Gupta A, Torres DC, Fu J, Kranz A, Stewart AF, Anastassiadis K.** 2014. The H3K4 methyltransferase Setd1a is first required at the epiblast stage, whereas Setd1b becomes essential after gastrulation. Development **141:**1022–1035. http://dx.doi.org/10.1242/dev.098152.
80. **Andreu-Vieyra CV, Chen R, Agno JE, Glaser S, Anastassiadis K, Stewart AF, Matzuk MM.** 2010. MLL2 is required in oocytes for bulk histone 3 lysine 4 trimethylation and transcriptional silencing. PLoS Biol **8:**e1000543. http://dx.doi.org/10.1371/journal.pbio.1000453.
81. **Gu B, Lee MG.** 2013. Histone H3 lysine 4 methyltransferases and demethylases in self-renewal and differentiation of stem cells. Cell Biosci **3:**39. http://dx.doi.org/10.1186/2045-3701-3-39.
82. **Herz HM, Garruss A, Shilatifard A.** 2013. SET for life: biochemical activities and biological functions of SET domain-containing proteins. Trends Biochem Sci **38:**621–639. http://dx.doi.org/10.1016/j.tibs.2013.09.004.
83. **Narayanan A, Ruyechan WT, Kristie TM.** 2007. The coactivator host cell factor-1 mediates Set1 and MLL1 H3K4 trimethylation at herpesvirus immediate early promoters for initiation of infection. Proc Natl Acad Sci U S A **104:**10835–10840. http://dx.doi.org/10.1073/pnas.0704351104.
84. **Schaffer P, Vonka V, Lewis R, Benyesh-Melnick M.** 1970. Temperature-sensitive mutants of herpes simplex virus. Virology **42:**1144–1146. http://dx.doi.org/10.1016/0042-6822(70)90364-8.
85. **Ottosen S, Herrera FJ, Doroghazi JR, Hull A, Mittal S, Lane WS, Triezenberg SJ.** 2006. Phosphorylation of the VP16 transcriptional activator protein during herpes simplex virus infection and mutational analysis of putative phosphorylation sites. Virology **345:**468–481. http://dx.doi.org/10.1016/j.virol.2005.10.011.
86. **Oh HS, Bryant KF, Nieland TJF, Mazumder A, Bagul M, Bathe M, Root DE, Knipe DM.** 2014. A targeted RNA interference screen reveals novel epigenetic factors that regulate herpesviral gene expression. mBio **5:**e01086-01013. http://dx.doi.org/10.1128/mBio.01086-13.
87. **Knipe DM, Smith JL.** 1986. A mutant herpesvirus protein leads to a block in nuclear localization of other viral proteins. Mol Cell Biol **6:**2371–2381.
88. **Showalter SD, Zweig M, Hampar B.** 1981. Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. Infect Immun **34:**684–692.
89. **Cliffe AR, Coen DM, Knipe DM.** 2013. Kinetics of facultative heterochromatin and polycomb group protein association with the herpes simplex viral genome during establishment of latent infection. mBio **4:**e00590-2. http://dx.doi.org/10.1128/mBio.00590-12.
90. **Wang Q-Y, Zhou C, Johnson KE, Colgrove RC, Coen DM, Knipe DM.** 2005. Herpesviral latency-associated transcript gene promotes assembly of heterochromatin on viral lytic-gene promoters in latent infection. Proc Natl Acad Sci U S A **102:**16055–16059. http://dx.doi.org/10.1073/pnas.0505850102.
91. **Cliffe AR, Garber DA, Knipe DM.** 2009. Transcription of the herpes simplex virus latency-associated transcript promotes the formation of facultative heterochromatin on lytic promoters. J Virol **83:**8182–8190. http://dx.doi.org/10.1128/JVI.00712-09.
92. **Orzalli MH, Conwell SE, Berrios C, Decaprio JA, Knipe DM.** 2013. Nuclear interferon-inducible protein 16 promotes silencing of herpesviral and transfected DNA. Proc Natl Acad Sci U S A **110:**E4492–E4501. http://dx.doi.org/10.1073/pnas.1316194110.
93. **Lee TI, Johnstone SE, Young RA.** 2006. Chromatin immunoprecipitation and microarray-based analysis of protein location. Nat Protoc **1:**729–748. http://dx.doi.org/10.1038/nprot.2006.98.